



Proceeding Paper

# Related Effects of Climate Change on Root System Dynamics and Symbiotic Activity in Local Bean Varieties (*Phaseolus vulgaris* L.) in the Humid Tropics of Costa Rica <sup>†</sup>

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## Abstract

Climate change poses an increasing challenge to tropical agriculture, particularly for heat-sensitive crops such as local varieties of beans (*Phaseolus vulgaris* L.). This study evaluated the effects of induced passive heat on the root architecture and symbiotic interactions of two local genotypes, *Tayní* and *Bruncas*, using Open Top Chambers under field conditions. Both varieties were included in the analysis and exhibited consistent qualitative responses to warming, with no contrasting cultivar-specific trends detected. Both varieties developed more roots and greater root area compared to the control, while mycorrhizal colonization increased up to 80% under warming. Soil temperature emerged as the main environmental factor influencing root expansion. These findings highlight the adaptive plasticity of local bean varieties under induced passive heat.

**Keywords:** *Tayní*; *Bruncas*; Open Top Chambers; abiotic stress



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## 1. Introduction

Climate change is reshaping agricultural systems worldwide through rising temperatures, altered rainfall patterns, and increasingly frequent extreme events, posing major challenges for tropical crop production. Recent studies on common bean varieties indicate that plant resilience to temperature stress can be strongly influenced by belowground biological interactions, highlighting the importance of biotic components in crop responses to climate warming [1]. Open Top Chambers (OTCs) have become a widely used approach to simulate moderate warming and assess plant physiological and morphological responses under field conditions, providing insights into how crops cope with future climate scenarios [2,3]. Beans (*Phaseolus vulgaris* L.), a key nutritional staple in Latin America and Africa, are particularly sensitive to heat stress due to their C3 photosynthetic pathway, which increases photorespiration and reduces productivity under elevated temperatures [4].

Despite their cultural and agronomic importance, local Costa Rican bean varieties remain understudied regarding their belowground responses to climate warming. Root systems play a critical role in plant adaptation by regulating water and nutrient acquisition and sustaining key symbioses such as nitrogen-fixing nodules and arbuscular mycorrhizae, both essential for resilience under stress [5,6]. Experimental evidence further shows that

increases in temperature can modify arbuscular mycorrhizal fungal communities and colonization dynamics, with important consequences for plant–microbe interactions and nutrient acquisition under climate change scenarios [7].

In the context of climate change, crop resilience increasingly depends on the capacity of plants to maintain functional belowground interactions under rising temperatures. Temperature increases can directly affect root physiology and indirectly reshape plant–microbe symbioses by altering carbon allocation, nutrient exchange, and microbial activity in the rhizosphere [4,8]. In Leguminosae, arbuscular mycorrhizal associations play a central role in enhancing nutrient acquisition, buffering abiotic stress, and supporting plant performance under suboptimal thermal conditions [4,5]. Therefore, understanding how moderate warming influences root system dynamics and mycorrhizal functioning is critical for evaluating the adaptive capacity of legume crops under future climate scenarios.

This study evaluates how moderate warming affects root structure, nutrient assimilation, and symbiotic interactions in two Costa Rican bean landraces using OTCs under field conditions. By integrating real-time abiotic measurements with structural and functional root assessments, the research aims to investigate the adaptive capacity of these genotypes and contribute to the development of climate-resilient strategies for tropical bean production.

## 2. Materials and Methods

The research was carried out at EARTH University's experimental fields in Guácimo, Limón, Costa Rica (10°12'02" N, 83°36'12" W) [2]. OTCs and ambient control were the two environmental conditions used to test the two native bean types, *Tayni* (red seeds) and *Bruncas* (black seeds). A total of 12 units each setting and 6 units per variety made up the total of 24 experimental units. Between 10 and 15 plants were included in each unit, and 10 of the plants were destructively sampled for root analysis.

OTCs were constructed from UV-resistant acrylic (3 mm) with dimensions of 0.85 m height, 1 m upper opening, and 1.5 m base width, producing passive heating between 1.9 °C and 5.0 °C relative to ambient conditions [8]. Microclimatic variables were monitored using SensorPush sensors (SensorPush Inc., Brooklyn, NY, USA) for air temperature and humidity, and Elitech RC-5 data loggers (Elitech Technology Inc., San Jose, CA, USA) with external probes at 15–30 cm depth for soil temperature.

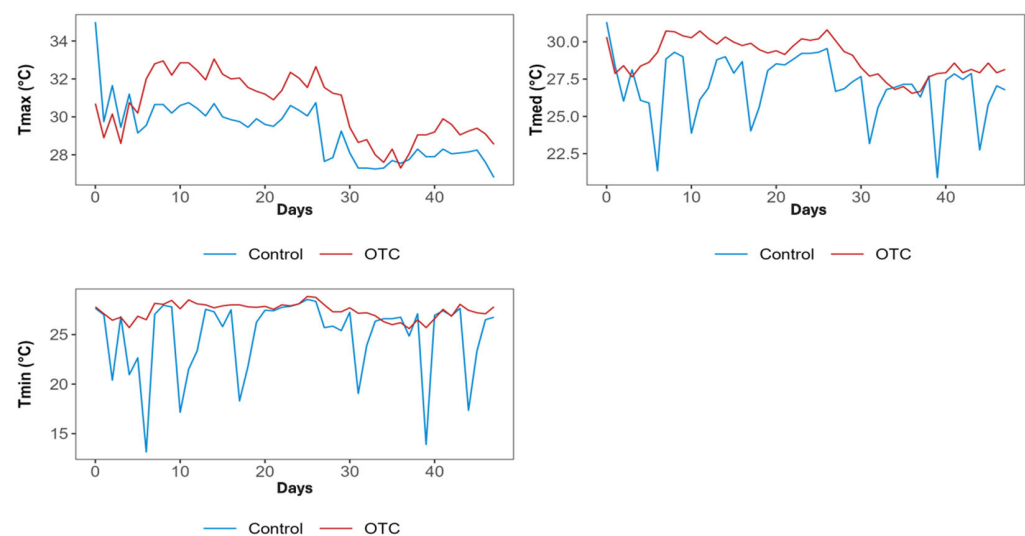
Root morphology (root number and surface area) was assessed using APS Assess 2.0 (APS Press, St. Paul, MN, USA) from cleaned, high-resolution root images. Functional traits included nitrogen content measured via the Dumas method using a C/N analyzer (Vario MAX CN, Elementar Analysensysteme GmbH, Langenselbold, Germany), nodule counts following [9], and arbuscular mycorrhizal colonization quantified through KOH clearing, Trypan Blue staining, and grid-line intersection microscopy [10]. Arbuscular mycorrhizal fungi were not identified to species level, and no molecular or taxonomic characterization of the fungal community was performed; therefore, mycorrhizal colonization was assessed exclusively as a functional indicator of symbiosis. Nodulation was quantified as the total number of visible nodules per plant at harvest. All plants were destructively sampled at the maturation stage, after crop harvest, and nodules were counted on the entire root system. Nodulation was therefore expressed on a per-plant basis and not normalized to root length or root surface area.

Statistical analyses were performed in R (version 4.3.2) using generalized linear models to test effects of genotype and environment on root traits, complemented by repeated-measures ANOVA for microclimatic differences and Tukey tests for pairwise comparisons ( $p < 0.05$ ).

### 3. Results and Discussion

#### 3.1. Soil Temperature Dynamics Under OTC and Control Conditions

Soil temperature exhibited a clear and consistent warming effect in the OTC treatment compared with the ambient control. Across the experimental period, maximum soil temperatures in the OTC exceeded those of the control by approximately 4 °C, while mean and minimum temperatures also remained higher and more stable. In contrast, control conditions showed strong daily fluctuations, including sharp drops in minimum temperature, which were largely absent in the OTC. The more stable thermal profile in the OTC demonstrates that the passive heating system effectively maintained elevated soil temperatures throughout the 50 days (Figure 1).



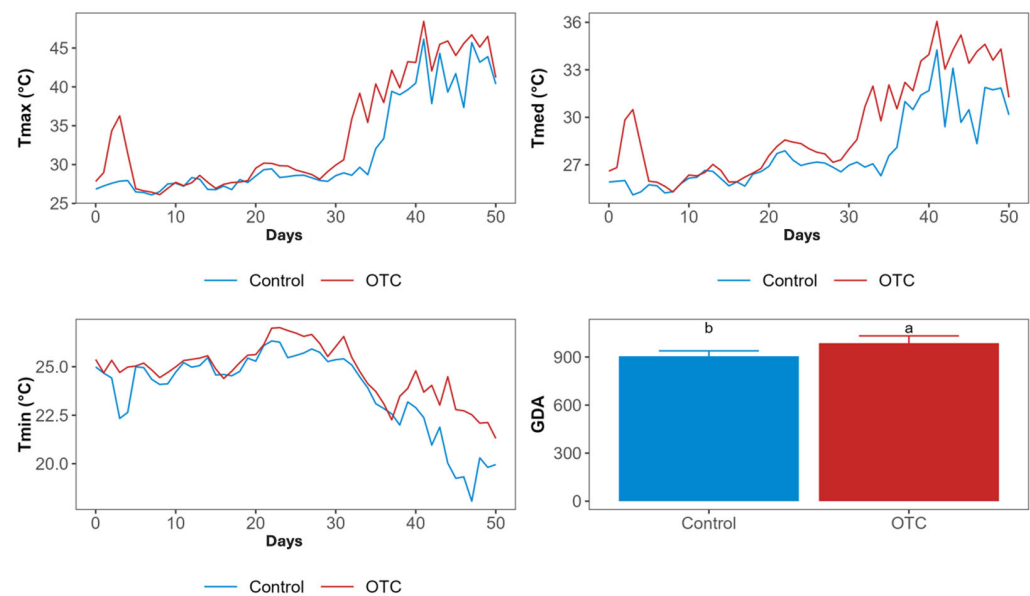
**Figure 1.** Soil temperature variation (maximum, mean, and minimum) over 50 days under Open Top Chamber (OTC) and Control conditions. Tmax: Daily maximum temperature; Tmed: Daily mean temperature; Tmin: Daily minimum temperature; OTC: Open Top Chamber.

These temperature patterns have direct physiological implications. The sustained heating and reduced variability in the OTC align with the expected behavior of passive heating structures described in global change research. Similar studies using OTCs reported 2–4 °C soil warming and reduced night-time cooling, consistent with our observations [3,11].

Since optimal soil temperatures for *Phaseolus vulgaris* root development typically range between 21–28 °C, the colder and more variable control environment may impose episodic metabolic stress [12]. Thus, the differences in subsequent root morphological and functional traits between treatments can be attributed, in part, to the contrasting soil thermal regimes demonstrated in our study.

#### 3.2. Ambient Temperature and Growing Degree Accumulation Under OTC and Control Conditions

Ambient temperature also showed a consistent warming trend in the OTC compared with the control environment. Maximum daily temperatures in the OTC were frequently 2–3 °C higher than in the control, particularly after day 20, when thermal divergence became more pronounced. Mean temperatures followed the same pattern, with the OTC maintaining elevated values and reduced fluctuations throughout the monitoring period. Minimum temperatures showed the clearest separation, as the control condition exhibited sharp night-time drops, whereas the OTC dampened these extremes (Figure 2). This reflects the buffering capacity of OTC structures, which reduce convective heat loss and maintain higher night temperatures, similar to previous reports in warming experiments [2].



**Figure 2.** Daily ambient maximum, mean, and minimum temperature under Control and OTC (Open Top Chamber) conditions over 50 days, and accumulated Growing Degree Days Accumulated (GDA). Tmax: Daily maximum temperature; Tmed: Daily mean temperature; Tmin: Daily minimum temperature. Different letters indicate statistically significant differences between treatments according to Tukey's post hoc test ( $p < 0.05$ ).

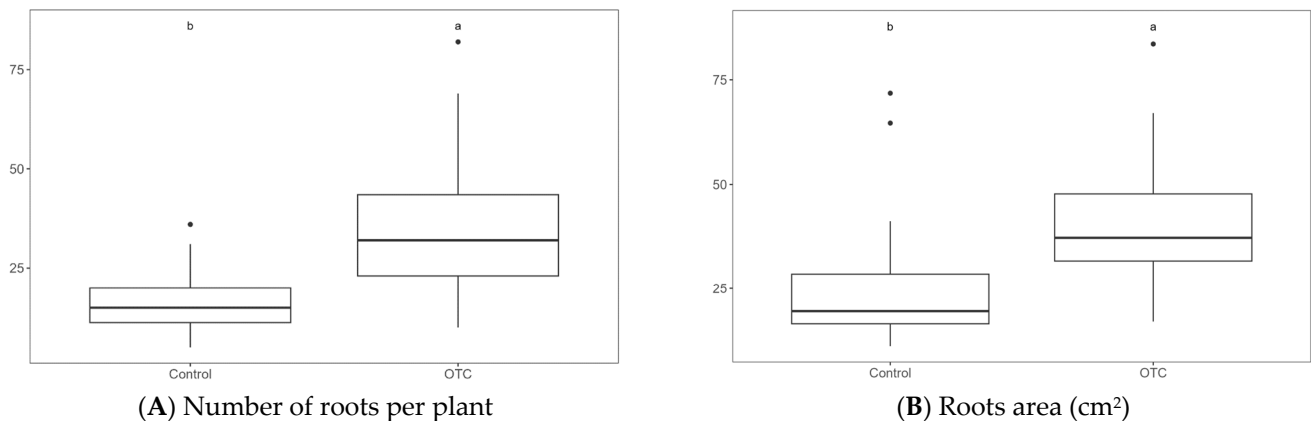
The cumulative effect of these thermal differences was captured in the GDA values, which were significantly higher in the OTC than in the control. Higher GDA indicates accelerated thermal accumulation and greater energetic input for physiological processes, including photosynthesis, respiration, and root activity. Warmer and more stable temperatures have been shown to enhance metabolic rates in *Phaseolus vulgaris* until reaching stress thresholds [2,4]. The stronger thermal load and reduced nocturnal cooling inside the OTC thus provide a clear abiotic basis for interpreting subsequent differences in plant growth, root development, and symbiotic behavior observed in this study.

### 3.3. Root Morphology Under Open Top Chamber and Control Conditions

Both root number and root surface area increased significantly under OTC conditions compared with the control. These responses were observed in both *Taynī* and *Bruncas* varieties, which displayed similar directional trends under OTC conditions; therefore, results are presented by treatment to emphasize the overall warming effect. Plants grown inside the warming chambers developed a markedly higher number of roots, with median values approximately double those of the ambient environment. Root surface area followed the same trend, showing a clear upward shift in the OTC, indicating enhanced root proliferation and spatial expansion. These results reflect a consistent morphological response to the elevated and more stable soil temperatures recorded in the OTC, which are known to stimulate root growth and branching in *Phaseolus vulgaris* (Figure 3).

From a physiological standpoint, warmer but not extreme soil temperatures can intensify root metabolic activity, increase meristematic division, and improve the capacity of plants to explore the soil profile. Previous studies have shown that heat stimulated root expansion is a common acclimation response in legumes under moderate warming, particularly when fluctuations in minimum temperature are reduced [13]. Elevated root surface area also enhances water and nutrient uptake potential and supports stronger symbiotic interactions, including rhizobial nodulation and mycorrhizal colonization [5]. Thus, the morphological improvements observed in the OTC not only represent a structural

adaptation to warming but provide the foundation for the functional responses reported later in this study.



**Figure 3.** Root morphology responses of the local beans varieties: (A) Number of roots per plant; (B) Root surface area (cm<sup>2</sup>) under Control and OTC conditions. OTC: Open Top Chamber. Each point represents an individual plant (biological replicate) sampled at the maturation stage. Boxplots show the median, interquartile range, and minimum and maximum values. Data were analyzed using generalized linear models, and pairwise comparisons between treatments were performed using Tukey post hoc tests ( $p < 0.05$ ). Different letters indicate statistically significant differences between treatments according to Tukey's post hoc test ( $p < 0.05$ ).

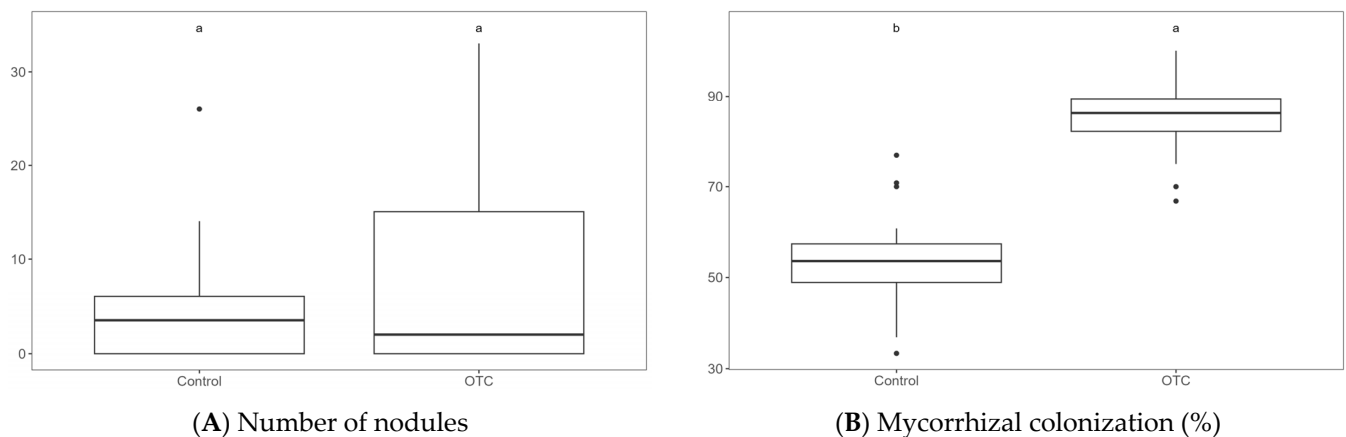
Comparable increases in root growth under moderate warming have been reported for *Phaseolus vulgaris* and other legume species, where warmer but non-lethal soil temperatures stimulate meristematic activity and root proliferation as an acclimation response [11]. However, the magnitude of root expansion observed in the *Tayní* and *Bruncas* under OTC conditions, together with the strong symbiotic responses reported below, suggests a particularly pronounced belowground plasticity in these local genotypes. These responses should also be interpreted in the context of OTC warming experiments, whose microclimatic performance and limitations have been comprehensively evaluated across environments [9].

### 3.4. Nodulation and Mycorrhizal Colonization Responses Under OTC and Control Conditions

The number of nodules per plant showed a tendency to increase under OTC conditions, although differences between treatments were not always statistically significant. Some plants grown inside the warming chambers developed higher nodule counts, suggesting that moderate temperature elevation does not inhibit symbiotic nitrogen fixation and may stimulate nodulation in certain individuals (Figure 4A). This trend is coherent with the observed increase in root number and surface area, which provides more potential infection sites for rhizobia. In addition, nodulation was quantified on a per-plant basis at the maturation stage and not normalized to root surface area, therefore, no inference can be made regarding nodulation density or symbiotic efficiency per unit root area.

Mycorrhizal colonization displayed a much stronger and significant response to warming. Plants exposed to OTC conditions exhibited markedly higher colonization percentages, with several individuals reaching 70–80%, compared with substantially lower values in the control environment (Figure 4B). This pattern was consistent across both bean varieties, with no evidence of opposing cultivar-specific responses to warming. In addition, this enhanced colonization suggests that moderate warming favors arbuscular mycorrhizal fungi by improving root growth, carbon allocation, and metabolic activity. Prior research confirms that warmer soils often enhance mycorrhizal functionality and can increase plant resilience to abiotic stresses such as heat and drought [6,14]. These results indicate that both

bean varieties maintain a plastic and responsive symbiotic system under passive heating, reinforcing their potential value for climate resilient agriculture.



**Figure 4.** (A) Number of nodules per plant under Control and OTC conditions. (B) Mycorrhizal colonization (%) under Control and OTC conditions. Nodules were counted per plant on the entire root system at the maturation stage. Each point represents an individual plant (biological replicate). Boxplots display the median, interquartile range, and minimum and maximum values. Statistical analyses were conducted using generalized linear models, with Tukey post hoc tests applied for pairwise comparisons between treatments ( $p < 0.05$ ). Different letters indicate statistically significant differences between treatments according to Tukey's post hoc test ( $p < 0.05$ ).

Although mycorrhizal colonization was evaluated as a functional response and fungal taxa were not identified, a plausible working hypothesis is that moderate warming may shift the activity or relative abundance of arbuscular mycorrhizal fungal groups with different thermal sensitivities. Such functional shifts in the mycorrhizal community, even without taxonomic resolution, could influence overall colonization levels and symbiotic functioning, thereby contributing to the enhanced mycorrhizal responses observed under OTC conditions. Testing this hypothesis would require future studies combining functional assessments with molecular or taxonomic characterization of the fungal community.

In Leguminosae, arbuscular mycorrhizal associations are particularly relevant because they complement biological nitrogen fixation by enhancing phosphorus uptake, improving nutrient balance, and increasing root absorptive capacity. Under increasing temperatures, these symbioses can play a central role in buffering thermal and nutritional stress by sustaining nutrient acquisition and supporting metabolic functioning. The strong increase in mycorrhizal colonization observed under OTC conditions therefore represents more than a structural response; it highlights a functional mechanism that may contribute to the resilience of local bean varieties facing moderate warming.

### 3.5. Carbon and Nitrogen Content in Roots Under Open Top Chamber and Control

Root chemical composition differed between thermal environments, with OTC plants exhibiting a lower carbon concentration and higher nitrogen content than the control. As a consequence, the C/N ratio decreased from 33.43 in the control to 26.38 in the OTC, indicating relatively greater nitrogen assimilation under warming. This pattern is consistent with the enhanced root growth and mycorrhizal colonization observed in the OTC, which can increase nutrient acquisition and promote allocation of nitrogen-rich compounds to support active metabolism (Table 1).

The lower root carbon concentration observed under OTC conditions may reflect increased carbon turnover rather than reduced carbon supply. Moderate warming is known to accelerate metabolic activity in plant roots, leading to higher growth and maintenance

respiration, which can decrease carbon concentration in root tissues even when overall plant growth is stimulated [10]. In legumes, assimilated carbon is often preferentially allocated to support active root development and symbiotic interfaces rather than structural carbon storage, particularly under favorable thermal conditions [4,11].

**Table 1.** Carbon (C), nitrogen (N), and C/N ratio in bean roots under Control and Open Top Chamber conditions.

| Ambient          | C (%) | N (%) | C/N   |
|------------------|-------|-------|-------|
| Open Top Chamber | 38.78 | 1.47  | 26.38 |
| Control          | 42.13 | 1.26  | 33.43 |

In addition, the markedly higher arbuscular mycorrhizal colonization recorded under OTC conditions likely represents an additional carbon sink, as host plants allocate photosynthates to sustain fungal symbionts in exchange for improved nutrient acquisition [12]. Together, these mechanisms provide a coherent explanation for the observed decrease in root carbon concentration alongside increased root surface area, nitrogen content, and symbiotic activity under warming.

Lower C/N ratios in roots are often associated with higher protein content and greater metabolic activity, particularly under moderate stress conditions that stimulate acclimation rather than damage. Studies in common bean and other legumes have shown that adequate nitrogen supply and efficient symbiotic functioning can partially offset the negative effects of elevated temperatures on growth and yield [2,4,8,12]. In our case, the reduced C/N ratio under the OTC suggests that, within the thermal range imposed, local bean varieties maintained or even improved their nitrogen-use efficiency, supporting the view that these genotypes possess functional plasticity that may be advantageous under future warming scenarios.

Across all measured variables, moderate passive warming consistently enhanced belowground performance in both local bean varieties. The OTC increased soil and ambient temperatures while reducing daily fluctuations, creating a more stable microenvironment for root activity. This thermal stability was reflected in the strong increases observed in root number, root surface area, and mycorrhizal colonization under OTC conditions. Although nodulation showed greater variability, several plants displayed higher nodule formation in the warming treatment, suggesting that symbiotic nitrogen fixation was not negatively affected within the temperature range imposed. The biochemical responses complemented the morphological and symbiotic patterns. Roots from plants grown in the OTC accumulated more nitrogen and showed a lower C/N ratio compared with the control, indicating more active nutrient uptake and metabolic activity under warming. Together, these results demonstrate that moderate temperature increases that are similar to projected climate trends for tropical regions can promote root development, enhance beneficial symbioses, and improve nitrogen acquisition in these local bean varieties. This integrated response reflects a notable degree of functional plasticity and suggests a potential resilience of these genotypes to future warming scenarios.

#### 4. Conclusions

Moderate passive warming induced through Open Top Chambers generated a consistently warmer and more stable environment that enhanced root growth, mycorrhizal colonization, and nitrogen accumulation in two local Costa Rican bean varieties. Both *Tayní* and *Bruncas* exhibited comparable qualitative responses to warming, indicating that the observed belowground plasticity reflects a shared adaptive capacity rather than cultivar-specific divergence under the conditions tested. The observed increases in root

surface area, symbiotic activity, and nutrient content indicate that these genotypes possess adaptive traits that allow them to maintain or even improve belowground functioning under elevated temperatures. These findings highlight the potential of local bean varieties as climate resilient resources for future agricultural systems and support the continued exploration of their physiological responses under changing environmental conditions.

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**Data Availability Statement:** The data presented in this study are available on reasonable request from the corresponding author.

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